

<http://www.pjbs.org>

**PJBS**

ISSN 1028-8880

**Pakistan  
Journal of Biological Sciences**

**ANSI***net*

Asian Network for Scientific Information  
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

## Fungal/Mycotic Diseases of Poultry-diagnosis, Treatment and Control: A Review

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**Abstract:** Fungal/mycotic diseases cause significant economic losses to the poultry industry either due to their direct infectious nature or due to production of mycotoxins, the secondary fungal metabolites produced in grains or poultry feed. Several fungi have created havoc in the poultry industry and some of them cause direct harm to human health due to their zoonotic implications. They are responsible for high morbidity and mortality, especially in young birds and cause stunted growth and diarrhea; and fatal encephalitis. Mycotic dermatitis is a possible health hazard associated with poultry houses. Mycotoxins are the leading cause of producing immunosuppression in birds, which makes them prone to several bacterial and viral infections leading to huge economic losses to the poultry industry. In comparison to bacterial and viral diseases, advances in diagnosis, treatment, prevention and control of fungal diseases in poultry has not taken much attention. Recently, molecular biological tools have been explored for rapid and accurate diagnosis of important fungal infections. Effective prevention and control measures include: appropriate hygiene, sanitation and disinfection, strict biosecurity programme and regular surveillance/monitoring of fungal infections as well as following judicious use of anti-fungal drugs. Precautionary measures during crop production, harvesting and storing and in feed mixing plants can help to check the fungal infections including health hazards of mycotoxins/mycotoxicosis. The present review describes the fungal pathogens causing diseases in poultry/birds, especially focusing to their diagnosis, prevention and control measures, which would help in formulating appropriate strategies to have a check and control on these unwanted troubles to the poultry producers/farmers.

**Key words:** Fungal disease, poultry, birds, aspergillosis, candidiasis, cryptococcosis, dactylariosis, histoplasmosis, mucormycoses, rhodotorulosis, torulopsis, white comb, diagnosis, prevention, treatment, control

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### INTRODUCTION

Fungal/mycotic infections are common in all kinds of poultry birds but are less prevalent as compared to bacterial and viral infections. Fungi are eukaryotic organisms, comprising of both yeasts and molds. Fungal diseases of poultry include Aspergillosis, Candidiasis, Dactylariosis, Cryptococcosis, Favus, Rhodotorulosis, Torulopsis, Mucormycoses, Histoplasmosis and Cryptococcosis. Out of these, the first two (Aspergillosis and Candidiasis) are having much importance and impact and the last two (Histoplasmosis and Cryptococcosis) have some zoonotic significance. Fungi produce disease in two ways viz. producing pathogenic signs and lesions

of disease by invading, harming and destroying body tissues of the host; and by producing some toxins known as mycotoxins (aflatoxins, ochratoxins, ergot, fusarium toxins etc.) in food grains and feed during crop production, harvesting and storage steps, the intake, consumption and subsequent intoxication of which produce disease, immunosuppressive condition and hampers production potential (Hubalek, 1978; Jand and Singh, 1995; Jand *et al.*, 2005; Kataria *et al.*, 2005; Dahlhausen, 2006; Dhama *et al.*, 2007, 2011a; Rai *et al.*, 2011; Singh *et al.*, 2012). Sporadic infections are common but sometimes they may take the form of outbreaks (Kunkle, 2003a; Saif, 2003; Shivachandra *et al.*, 2004; Jand *et al.*, 2005; Chauhan and Roy, 2008;

Dhama and Mahendran, 2008; Turner *et al.*, 2009; Dhama *et al.*, 2011a; Singh *et al.*, 2012). Seasonal variation plays important role in spread of fungal infections. Predominance of infection in closed housing during summer and the presence of fungi in the poultry litter material during autumn make the eradication difficult (Soliman *et al.*, 2009). Fungal diseases are assuming new importance because of the inappropriate use of antibacterial that eliminate the natural beneficial microflora which otherwise suppress the growth of fungi (De Lucca, 2007). The fungal pathogens mainly target the respiratory and nervous system of poultry and cause specific pathological changes in the host characterized by inflammation, lesions and sickness leading to death (Shivachandra *et al.*, 2004). Chronic exposure to fungal spores produces allergic responses in sensitized birds resulting in illness and decreased productivity. Fungal infections require appropriate attention in terms of timely diagnosis and effective treatment regimens to be followed. Advances in the treatment and control of bacterial and viral diseases of poultry have been outstanding in the recent years but the situation is not so good in case of fungal infections and thus is a matter of concern (Jand and Singh, 1995; Kunkle, 2003b; Dhama and Mahendran, 2008; Dhama *et al.*, 2008, 2011a). The discovery of mycophages (fungal viruses) have increased the expectation as they can be exploited as a means of biological control and even to explain the variation in antibiotic production and instability of fungal strains (Ghabrial, 1980). The present review describes the various fungal diseases of poultry with emphasis on their diagnosis, treatment, prevention and control.

**Aspergillosis (brooder's pneumonia):** Aspergillosis, commonly known as brooder's pneumonia, is caused mainly by *Aspergillus fumigatus*, most pathogenic fungi affecting poultry (Arne *et al.*, 2011) but *A. flavus* has also been the culprit associated with many cases. Respiratory infection by *Aspergillus* spp. has been reported in almost all types of poultry birds viz., layer cockerels (Throne Steinlage *et al.*, 2003), broilers (Martin *et al.*, 2007), growers (Zafra *et al.*, 2008) and turkey poults (Olias *et al.*, 2010). Turkeys are having higher susceptibility to aspergillosis when compared to chickens. *A. fumigatus* infection occurs more frequently in poultry as the spores of this pathogen species are smaller than those of other *Aspergillus* spp. (Richard and Thurston, 1983; Arne *et al.*, 2011). Other *Aspergillus* spp. that may affect birds adversely are *A. terreus*, *A. glaucus*, *A. nidulans* and *A. niger* (Beernaert *et al.*, 2010; Dhama *et al.*, 2012). *Aspergilli* can be isolated from environmental samples and are worldwide in distribution.

Spores of this fungal pathogen are resistant in nature. Poultry birds coming in contact with the spores through contaminated feed or litter gets affected after inhaling the spores. The predisposing factors for flaring spore generation and dissemination in the air/environment include warm environment, humidity, poor ventilation and sanitation along with long term storage of feed (Tell, 2005; Khosravi *et al.*, 2008). The disease develops in brooder stages in chicks as well as passerine birds, especially below three days of age (Pokras, 1988; Chauhan and Roy, 2008; McMillan and Petrak, 1988). Exposure generally occurs by inhalation of spores, which often originate from infected eggs that are opened. Chicks may get infection in the hatcheries itself as by the release of large number of spores in the environment and contaminate hatch mates (Oglesbee, 1997). Aspergillosis is a necrotizing and granulomatous cavity disease of the lungs with hematogenous spread (Ganguly *et al.*, 2011). High humidity and moderate temperature conditions contributes significantly towards the occurrence and spread of aspergillosis (Dhama *et al.*, 2008), thereby facilitating seasonal occurrence of the disease in waterfowls with higher incidences in spring and autumn. Particularly, crippled and malnourished captive birds suffer individually. Contaminant like lead acts as a precipitating factor, especially in geese (Wobeser, 1997; Kapetanov *et al.*, 2011).

Aspergillosis primarily causes high morbidity and mortality especially in young chicks/birds (Redig, 2005; Arne *et al.*, 2011). The disease occurs in two main forms-acute and chronic. Acute aspergillosis (brooder's pneumonia) occurs as a result of inhaling high number of spores, wherein severe disease outbreaks in young birds are characteristically observed. Morbidity and mortality are high (70-90%) in it and can be seen within 24-48 h of infection. Chronic form occurs sporadically and is the generally observed in adult breeder birds (particularly turkeys) or occasionally in an adult flock causing significant economic losses. This form is associated with immune suppression (Vanderheyden, 1993). Proteases and toxic secondary metabolites secreted by the fungus contribute to virulence (Tekaiia and Latge, 2005) along with gliotoxin, a highly immunosuppressive mycotoxin. Air sacculitis is observed when concentrations of gliotoxins exceed 20- 70  $\mu\text{g g}^{-1}$  in poultry feedstuffs and in tissues of turkeys (Pena *et al.*, 2010). However, the true virulence factor is indecisive due to environmental and clinical conditions inducing the disease condition in susceptible birds. The pathogen clearance mechanisms in poultry rely on mucous-covered ciliated epithelial cells lining the upper respiratory tract (Reese *et al.*, 2006). Lytic changes are observed in the epithelium of the upper

airway due to inhalation of conidia by even short duration of exposure to contaminated environment (Nganpiep and Maina, 2002). The initial physical barriers can be broken apart by the conidia of *A. fumigatus* that are small enough (2-3 µm in diameter) to deeply penetrate the respiratory system of birds.

Affected birds may show gasping along with fever, foetid diarrhea and rapid loss of condition with convulsions occurring sometimes. Sub-acute form develops within 8-10 days in birds upto 2 weeks of age with acute signs often present in a milder form together with anemia. Respiratory rattle may be observed. Faeces may also become yellowish (Reece *et al.*, 1986; Richard, 1997; Atasever and Gumussoy, 2004; Dhama *et al.*, 2011a). Yellow coloured pin point lesions are visible in lungs and air sacs, which can be seen through naked eyes and may range from miliary to larger granulomatous foci. Sometimes small yellow green granular fungus growth is observed in all the body cavities with dry consistency of lungs. Walls of air sacs may thicken and bronchioles may be filled with suppurates. Mycelial growth may extend into blood vessels from where they disseminate. Granulomas can develop in multiple organs (Calnek *et al.*, 1997; Fraser *et al.*, 1991; Shivaprasad, 2000). Distorted fruiting heads of the *Aspergillus* can be found in air sacs. Turkeys suffer from chronic disease terminating in impedence of pulmonary granulomas, causing right ventricular dilatation and ascites. Necrotic granulomatous dermatitis and cutaneous aspergillosis are reported in chicken and pigeons, respectively (Vanderheyden, 1993, Julian and Goryo, 1990; Nardoni *et al.*, 2006; Cacciuttolo *et al.*, 2009; Beernaert *et al.*, 2010).

Large number of spores are found in wet litter and gets aerosolized when the litter dries off. Biphasic mortality pattern is observed and in acute cases mortality range between 5-50% during initial 1-3 weeks of age. Survivors often develop chronic disease due to pulmonary insufficiency or neurological fungal metastasis and may become lethargic and stunted (Calderone and Fonzi, 2001; Beernaert *et al.*, 2010). Dyspnoea is common in neonates during first 3-5 days as evidenced by open mouth breathing (gasps) due to progressive airway obstruction. There may be affection of eyes leading to deposition of cheesy materials (in turkeys) and blindness along with Central Nervous System (CNS) abnormalities including torticollis (Dyar *et al.*, 1984; Jensen *et al.*, 1997; Steinlage *et al.*, 2003; Dhama *et al.*, 2011a).

Inhalation of large number of conidia from contaminated feed or litter affects epithelium of conjunctiva and respiratory tract initiating granulomas which causes pulmonary aspergillosis (Sauter *et al.*, 1981;

Lugauskas *et al.*, 2004). Ophthalmitis occasionally occur in turkeys upon experimental infection, wherein eyes may be cloudy with retinitis, iridocyclitis and secondary involvement of the remainder of eye (Akan *et al.*, 2002). Necrotic foci in the cerebrum or cerebellum cause encephalitis. Turkeys are quite sensitive to oral doses of necrotizing gliotoxin (immunosuppressive and cytotoxic), produced by various isolates of *A. fumigatus* and inhibit transformation of blood lymphocytes (Richard *et al.*, 1994, 1996; Richard and DeBey, 1995; Peden and Rhoades, 1992). Birds over 5 days of age show complete lesions along with uniform pinhead sized yellowish nodules in lungs. Air sacs become thickened and cloudy having yellowish plaques. Necrotic foci may occasionally be seen in the visceral organs (Okoye *et al.*, 1989; Steinlage *et al.*, 2003; Cacciuttolo *et al.*, 2009; Singh *et al.*, 2009). Rapid death due to aspergillosis can flare up avian influenza even though there is no connection between the two diseases and this requires laboratory attention to distinguish these two diseases (Kradin and Mark, 2008).

**Diagnosis:** Non-specific signs are common making diagnosis difficult (Dahlhausen *et al.*, 2004). Individual test does not provide reliable diagnosis and therefore confirmatory diagnosis requires disease history, clinical presentation, blood biochemical profile, serology, radiographic changes along with endoscopy and cultural examination of the fungus (Jones and Orosz, 2000). Stressful events are some adverse environmental factors and/or an immunosuppressive condition or treatment (Jenkins, 1991). Chronic debilitation, voice change and exercise intolerance also induce stress (Oglesbee, 1997). The clinical signs depend on the form of the disease and involvement of organ (Jones and Orosz, 2000), thereby requiring the disease to be differentiated from other systemic diseases of respiratory tract (Jenkins 1991; Jones and Orosz, 2000). Results of haematology and plasma biochemistry are better diagnostic indicators (Jones and Orosz, 2000). Serological tests include counter-immunoelectrophoresis, agar gel immunodiffusion and enzyme-linked immunosorbent assays. However, negative serological test results do not rule out aspergillosis; and positive tests must be backed up by other disease evidences (Peden and Rhoades, 1992; Brown and Redig, 1994; Redig *et al.*, 1997; Le Loch *et al.*, 2005; Arca-Ruibal *et al.*, 2006; Cray *et al.*, 2006, 2009a, b). Although, radiographs may not be helpful, but lateral and ventrodorsal views of a bird suspected for aspergillosis can give some indication and in absence of anaesthesia, standing or perching lateral as well as dorsoventral views are helpful (Jones and Orosz, 2000). Endoscopy of the abdominal air sac can reveal a diffuse cloudiness or white

or yellow plaques covered with green gray pigmented mould. Samples for culture and cytology should be taken directly with biopsy forceps or via air sac lavage (Jenkins 1991; Taylor, 1993; Oglesbee, 1997).

On necropsy, the granulomatous foci having varying degree of colour can be noted in chronically ill patients (Jenkins 1991; Vanderheyden, 1993). Acute aspergillosis causes numerous miliary granulomatous foci (McMillan and Petrak, 1989; Jenkins, 1991). Definitive diagnosis requires demonstration of the organisms by cytology or histopathology and subsequent identification by culture (Dahlhausen *et al.*, 2004). Isolation of the fungus alone does not confirm the infection status because *Aspergillus* organisms are ubiquitous contaminants (Jensen *et al.*, 1997; Flammer and Orosz, 2008). However, plentiful culturing from any organ should be considered for diagnosis, but a negative culture also can not rule out *Aspergillus* infection (Redig, 2005; Jensen *et al.*, 1997). Brain and heart along with organs of respiratory system like larynx, trachea and lungs are important for histopathological examination. Microscopic lesions can be suggestive but not helpful in species identification because *in vivo* hyphae of hyaline filamentous fungi are very similar and their *in situ* manifestations are not pathognomonic (Kaufman *et al.*, 1997; Tekaia and Latge, 2005; Cray *et al.*, 2009a). Thus, immunohistochemistry usually can provide confirmatory diagnosis, although few reports are only documented using monoclonal or polyclonal antibodies for diagnosing aspergillosis in birds (Carrasco *et al.*, 1993; Jensen *et al.*, 1997; Beytut *et al.*, 2004; Beytut, 2007).

Polymerase Chain Reaction (PCR) including real-time PCR assay is a valuable diagnostic tool. PCR based cloning and sequencing of Internal Transcribed Spacer (ITS) have been attempted successfully. Sophisticated techniques like Nucleic Acid Sequence Based Amplification (NASBA) and Molecular Beacon (MB) technology have increased the rapidity of diagnosis of this important pathogen (Calderone and Fonzi, 2001; Dhama and Mahendran, 2008; Saleemi *et al.*, 2012; Dhama *et al.*, 2011a, 2012; Zhao and Perlin, 2013).

**Prevention and control:** For prevention of aspergillosis, stress factors and exposure to spores need to be minimized along with adopting strict hygiene and sanitation measures in brooder and hatchery (Wright *et al.*, 1960; Chute and Richard, 1991; Beernaert *et al.*, 2010). Dirty, broken and potentially contaminated eggs must be eliminated before setting in the incubator. An effective fungicide should be applied inside the setter soon after transfer of hatching eggs is

completed (Wind and Yacowitz, 1960). Feed with low moisture content should be given and the litter should be kept dry. Screened and elevated platforms help to prevent turkeys from picking up molds from feed containers and waterer fountains. Proper drainage is necessary to prevent water logging (Chute and Richard, 1991). Maintain good ventilation, hygienic and stress-free environmental conditions inside the poultry farm. A good litter management practice needs to be followed and in between two flocks, treatment of new litter with antifungal agent is mandatory to prevent the disease (Richard *et al.*, 1984; Shivachandra *et al.*, 2004). Feeders should be kept dry and clean to limit the fungal development (Powell *et al.*, 1994; Akan *et al.*, 2002; Kunkle, 2003a). Affected and ill birds should be removed and culled/destroyed. Both conventional and supportive treatment are required. In mild form of disease, treatment is fruitful but when lesions are moderate to severe involving lungs and air sacs, therapy is often not successful even after combination of drugs are used. Various drugs like amphotericin-B, 5-fluorocytosine, ketoconazole can be used to control the disease (Dhama *et al.*, 2012). Treating litter with Nystatin and Copper sulphate can reduce mold content (Dyar *et al.*, 1984). Copper sulphate at 60 g quintal<sup>-1</sup> of feed for 6 days is effective for treatment of aspergillosis. In outbreaks, drinking water with 1:2000 aqueous solution of copper sulphate needs to be provided. Tetracycline at 200 mg L<sup>-1</sup> of drinking water should be given for 5 days to treat aspergillus infections in chicks. Other drugs like eniconazole and fungicidin have also been tried on experimental basis (Babras and Radhakrishnan, 1967; Arne *et al.*, 2011).

**Candidiasis (moniliasis, thrush or sour crop):**

Candidiasis otherwise known as thrush is a fungal disease caused by yeasts of the genus *Candida* having nearly 200 species (Odds, 1994). Among them, six are most frequently isolated. While *C. albicans* is the most abundant and significant species, *C. tropicalis*, *C. glabrata*, *C. parapsilosis*, *C. krusei* and *C. lusitanae* have also been implicated as causative agents. Susceptible hosts include domestic poultry, water fowls and wild birds (Tiwari *et al.*, 2011). Unhygienic atmosphere and secondary debilitating conditions result in both superficial and deep infections. Involvement of the digestive tract is common in young birds as compared to older birds. Increased virulence of the fungus plays a vital role in establishing the disease (Chute, 2001; Jungherr, 1933). *C. albicans* is an asporogenous and pseudomycelial dimorphic yeast having fermentation capability. It grows on ordinary media over a wide range of pH and temperature. Budding yeast forms

(blastospores) are 3-4 µm on epithelial surfaces whereas branching septate hyphae or pseudohyphae are 3-5 µm diameter in deeper tissues. It can utilize ammonia but not nitrate; nitrogen and most strains need growth factor biotin to be supplemented for their growth (Hubbard *et al.*, 1986; Novak *et al.*, 2003). Endogenous form of the disease is most common due to frequent presence of organisms in gastrointestinal tract and nature. *C. albicans* is isolated from environmental sources less commonly than other *Candida* species suggesting its adaptation to be parasitic, not saprophytic way of life. Especially in psittacine birds, key entry and multiplication site is the nasal cavity (Winner and Hurley, 1964; Balish and Phillips, 1966; Tsai *et al.*, 1992; Fulleringer *et al.*, 2006).

Transmission of *Candida* mainly occurs via fecal contaminated feed and water. *Candida* spp. may become part of the inhabitant flora of the mouth, esophagus and crop. Litter from poultry houses and game bird areas, waste and disposal areas contaminated with human waste are suggested as potential sources for exposure to *Candida* introduction (Bauck, 1994; Oglesby, 1997; Odds, 1988). Risk factors, which predisposes to candidiasis and aggravate disease include malnutrition, vitamin D deficiency, poor hygiene, prolonged use of antibiotic suppressing normal bacterial flora, stress an immunosuppressive diseases (Campbell, 1986; Kollias, 1986; Velasko, 2000) Recognized virulent factors of *C. albicans* include adhesins having affinity for the fibronectin on the cell surfaces, yeast forms cause tissue damage, phospholipase concentrated in hyphal tips may enhance invasiveness, the mycelial phase of *C. albicans* facilitates penetration of the fungus into tissues, cell wall glycoprotein has an endotoxin like activity, (Meunier-Carpentier *et al.*, 1981, Ruchel *et al.*, 1983; Macdonald, 1984; Ruchel, 1984). Neuraminidase, proteases, chitin, mannoprotein and lipids are other virulence factors. Phenotypic switching in *C. albicans* may facilitate evasion of host defense mechanisms. Systemic infection may occur when the fungus spread via haematogenous route after vascular invasion by hyphae or pseudohyphae. Inflammatory responses predominantly involve neutrophils and granulomatous lesions, but are rare (Calderone and Fonzi, 2001; Schaller *et al.*, 2005; Antony *et al.*, 2009).

Birds under 3 weeks of age are more susceptible to candidiasis. Affected poultry show poor and stunted growth, depression diarrhea and dehydration which that are altogether responsible for direct mortality (Chute, 1997; O'Meara and Witter, 1971; Bauck, 1994). Clinical signs are observed only in severely affected individual birds with superficial oral or crop infections. In

rare cases there is systemic invasion wherein neurological, renal or intestinal involvements become evident (Chute, 1997; Oglesby, 1997; Velasko, 2000).

Lesions are usually confined to the upper digestive tract. Yeasts proliferate on the surface and hyphae or pseudohyphae invade superficial epithelial layers, causing hyperplasia and pseudomembrane or diphtheritic membrane formation, grossly appearing as multifocal to confluent mats of cheesy material in the crop, but less frequently in the esophagus and pharynx (Mayeda, 1961; Velasko, 2000). Round raised ulcers or 'Turkish-towel' appearance in the mucosa are commonly observed (Bauck, 1994; Schmidt *et al.*, 2003). The membranous mass adhering to the surface of the crop cannot be easily removed. Other areas of the upper digestive tract develop false membranes resembling like that during diphtheria and contain considerable necrotic tissue. Erosion of the lining of the proventriculus and gizzard along with intestinal inflammation are commonly observed (O'Meara and Witter, 1971; Odds, 1988; Chute, 1997; Bethea *et al.*, 2010).

**Diagnosis:** For demonstrating hyphal forms of the yeast in the tissue, diagnosis based on the lesions, histopathology and microscopic examination of a digested smear are important. Cultural colonies of *Candida* appear as white to ivory colour and smooth having a yeasty smell. *C. albicans* can be isolated from faeces, crops, gizzards, lungs and livers. Isolation is done by embryo inoculation test via chorioallantoic membrane (CAM). Fifty percent of embryos may die between 48 and 72 h. Advent in molecular diagnostics generating tools like PCR-RFLP has made the diagnosis easier and confirmatory (Ayatollahi Mousavi *et al.*, 2007; Tiwari *et al.*, 2011).

**Treatment and control:** As the organism has broad host range, cages, equipments and other materials in contact with infected birds should be disinfected without any delay. Cleanliness, adequate hygienic/disinfection measures, proper managemental care and vitamin A supplementation are vital for disease prevention. Excessive use of antibiotics and other stressors must be avoided (Bauck, 1994; Chute, 2001; Dhama *et al.*, 2003). Treatment of candidiasis should take care of the predisposing conditions, risk factors or infections. Improved diet, husbandry and care can minimise the severity of infection and subsequent lesions and losses. Addition of vinegar to the drinking water acidifies the gastrointestinal contents, which is unfavourable for fungal growth. Addition of chlorhexidine in the drinking water helps to prevent overgrowth of *Candida* in poultry

flocks or nurseries (Underwood *et al.*, 1956; Underwood, 1955; Smith, 1987). However, immune suppression associated with the overuse of disinfectants need to be taken care of. Feeding of birds with diets low in simple carbohydrates (i.e., grains and sprouts) is advocated over seeds and sweets (Velasko, 2000). Invasive and well established infections require the use of anti-fungal agents such as Nystatin, azoles (fluconazole or itraconazole), or amphotericin B. For control, nystatin (100 g ton<sup>-1</sup>) or copper sulphate (2-3 lbs ton<sup>-1</sup>) to the feed for 7-10 days is prescribed (Flammer, 1993, 1994; Rupley, 1997; Velasko, 2000; Tiwari *et al.*, 2011). Suboptimal management conditions need to be avoided to prevent flaring up of the disease. Ideal practices like continual use of mold inhibitors in the feed, proper feed storage and handling practices, regular cleaning and sanitizing of the watering system and periodic stirring and/or replacement of wet litter areas are essential elements for disease prevention. Chlorine bleach added to the drinking water at 5 parts per million (ppm) is quite effective (Janmaat and Morton, 2010).

**Dactylarioris (mycotic encephalitis):** Dactylarioris is caused by a dematiaceous and thermophilic fungus-*Dactylaria gallopava* that affects young chicks (Georg *et al.*, 1964; Ranck *et al.*, 1974; Shane *et al.*, 1985). It grows well at 25-35°C with optimal temperature being 45°C and low pH (<5) conditions. Spores spread after getting released into air (Tansey and Brock, 1973; Waldrip *et al.*, 1974; Randall *et al.*, 1981). Birds become infected on inhaling spores but the disease is produced by angio-invasion and hematogenous spread to CNS. Birds between initial 1-5 weeks of age are susceptible. Mortality during disease outbreak ranges between 3-20%, mainly due to neurological disease (Blalock *et al.*, 1973; Waldrip *et al.*, 1974; Shane *et al.*, 1985). Torticollis and in-coordination due to paresis are observed in infected poults. Sometimes ocular lesions results into blindness. In rare cases pulmonary granuloma causes dyspnoea as observed in aspergillosis (Randall *et al.*, 1981; Sonne *et al.*, 2012). Hematogenous spread of spores to brain leads to development of lesions characterized by yellow or gray coloured meningeal or encephalitic necrotic lesions that are more common in cerebellum or caudal cerebral cortex (Blalock *et al.*, 1973; Salkin *et al.*, 1990). Clinical signs and gross lesions are not specific, making diagnosis difficult. Rapidly progressing nervous symptoms can also be seen in young birds with vitamin E deficiency (encephalomalacia) or other bacterial (meningitis) and viral (New castle disease) infections. Microscopically brain lesions are reflected in pigmented 2 µm diameter hyphae and large number of giant cells

(Blalock *et al.*, 1973; Ranck *et al.*, 1974). Sabraud Dextrose Agar (SDA) with suitable antibiotics and incubation at 45°C is suitable for fungal isolation from brain samples. Colonies produce brown color pigment diffusing into the surrounding medium and have characteristic diploid conidia (Ranck *et al.*, 1974). Effective treatment does not exist for dactylarioris. So, avoiding exposure to moldy litter, especially that with heat treatment is the only means of prevention (Kunkle, 2003b).

**Rhodotorulosis (mycotic dermatitis):** Rhodotorulosis is caused by pink yeast *Rhodotorula*, the yeast cells common contaminants and are infrequently associated with disease conditions (Vazquez, 2011). The fungus has been isolated from poultry litter and pigeon faecal droppings and is of public health concern. *R. glutinis* produces dermatitis in broiler chicken, while *R. mucilaginosa* cause dermatitis of feathers (Beemer *et al.*, 1970; Hubalek, 1978; Chauhan and Roy, 1996; Alvarez-Perez *et al.*, 2010). This yeast predominantly associated with trachea of fowls and has even been isolated from digestive organs (crop) along with *Aspergillus fumigatus* and *A. flavus*. Birds die suddenly with crop highly distended and filled with feed. Columbia agar with sheep blood (5%) or SDA with chloramphenicol supplementation are ideal for *Rhodotorula* isolation. Fungal isolates can be identified according to substrate accumulation profile and can be further confirmed by skin biopsy (Page *et al.*, 1980; Aruo, 1980; Grewal and Brar, 1987; Zaas *et al.*, 2003; Serena *et al.*, 2004; Tuon and Costa, 2008).

**Favus (white comb):** Favus is caused by *Microsporium gallinae* (Megnin) (*Trichophyton gallinae*), *Trichophyton simii*, *Microsporium gypseum* (Fonesca and Mendoza, 1984; Hubalek, 2000; Grunder *et al.*, 2005). This disease is not of much economical importance, occurs sporadically and as is seen associated to demographic poverty. The fungal spores enter via unbroken cutaneous surface during initial phase of infection, germinates in and around the hair follicle and shaft (seldom) (Kane *et al.*, 1997). Lesions are observed on featherless skin areas like comb, wattle and shanks; initially appearing as few grayish/yellowish cup like spots. They increase in size and coalesce to make a wrinkled crust, which is mostly dry and scaly appearing like honeycomb about the size of a pea. Feathered skin may develop lesions of depression around follicles (favus cup), systemic signs are not observed. Spread of infection occurs in birds by direct contact or via contaminated fomites (Londero *et al.*, 1969; Droual *et al.*, 1991; Saif, 2003). Favus is diagnosed by demonstration of the fungi in the smears. *Trichophyton*

gets easily cultured on Saboraud's glucose agar. Skin scrapings should be washed in 70% alcohol prior to attempting for cultural isolation (Bradley *et al.*, 1993; Saif, 2003). Microscopic examination is performed with the skin scab examination on a glass slide with potassium hydroxide solution (20%) and heated until appearance of a few bubbles; subsequently it is examined for presence of fungi. Staining of the fungus can also be done with 10% Parker Superchrome 51 pen ink in sodium hydroxide which demonstrates the presence of fungus. Replacement of the birds with new stock need to be made with disease free birds (symptom/lesion free). Proper segregation isolation procedures need to be followed to avoid introducing the disease into a healthy flock and to have check on its spread amongst the birds. If necessary, birds should be culled and slaughtered. Dipping of the birds in 0.5% pentachlorophenol or 5-bromosalicyl-4-chloramide, a multi-fungine ointment, or Ayurvedic 'Himax' ointment (Indian Herbs Research and supply Co.) is useful in external application (Chauhan and Roy, 2008).

**Torulopsis infection:** *Torulopsis glabrata*, the fungus responsible for causing *torulopsis* in poultry, is a haploid and non-dimorphic yeast. The disease is rarely seen in poultry and often is a problem in immunocompromised birds. Pathogenicity of the fungal agent is dependent on the epithelial adhesion genes, characteristically related with biofilm formation (Turner *et al.*, 2009). Liver gets enlarged in this disease condition and reveals yellowish-white and well defined nodules of variable size. Round fungal bodies with characteristic budding are observed in smear or section examination on fungal staining. Clinical signs observed are dullness, loss of appetite, ruffled feathers, etc. Identification is based on isolation of the causative fungus but cultural isolation may take much time. Azoles viz. fluconazole, ketoconazole etc are effective drugs of choice (Walker and Ayres, 1959; Fidel *et al.*, 1999; Chauhan and Roy, 2008).

**Mucormycosis:** Chickens are less susceptible to mucor infection; pneumonic lesions may be caused by *Mucor resimosus* or *M. chorimbifer*, while few species may cause infection in the eyes and vertebrae (Migaki *et al.*, 1970; Chauhan and Roy, 2008). *Mucor*, *Penicillium* and *Aspergillus* infections can occur through contaminated litter. In advanced cases, there is frequent involvement of the sinuses, brain and lungs. The infection can spread to gastrointestinal tract, skin and other organs. The most common types of the disease conditions are oral and cerebral mucormycosis (Spellberg *et al.*, 2005; Auluck, 2007). Contaminated litter need to be removed to effectively control the disease.

Presumptive diagnosis requires biopsy examination of the affected tissue, while examination of swabs of tissue or discharges is generally untrustworthy. Administration of one table-spoonful of 33% potassium iodide solution in drinking water per nearly 200 birds or antifungal drugs is helpful (Dawson *et al.*, 1976; Steinlage *et al.*, 2003; Dahlhausen, 2006).

#### Avian associated zoonotic fungal diseases

**Cryptococcosis:** Cryptococcosis, also known as Torulosis, Yeast meningitis, Busse-Buschke's disease and European blastomycosis), is caused by *Cryptococcus neoformans* that affects animals including poultry and humans (Singh and Dash, 2008; Dhama *et al.*, 2011b). Strains differ in virulence and immune status of the host is crucial (Velasko, 2000). The incubation period is probably in weeks. Cryptococcosis is not considered as true zoonoses. Fungus grows in soils with avian manures and thus causes an indirect public health implication. Infections in birds are rare. *C. neoformans* has been isolated from the feces of canaries (26%), carrier pigeons (18%), budgerigars (2%) and psittacine birds (1%), apart from domestic poultry (Saremi *et al.*, 2004; Singh and Dash, 2008). Humans acquire cryptococcosis from exposure to old pigeon nests or droppings (Cafarchia *et al.*, 2006; Abdel-Razik, 2007; Rosario *et al.*, 2008). Transmission occurs via inhalation and occasionally by ingestion and disease is usually chronic (Nosanchuk *et al.*, 2000; Malik *et al.*, 2003; Rosario *et al.*, 2008; Dhama *et al.*, 2011b). Clinic signs in humans include meningitis pulmonary infection, dyspnoea, chest pain, coughing, fever and malaise. Diarrhoea, weight loss, anaemia, headache, paralysis, stiff neck and visual disturbances may occur (Velasko, 2000; Dhama *et al.*, 2011b). Diagnosis requires culturing the organism in SDA media, observing histopathological changes, but with absence of inflammatory reaction. Cryptococci capsule develop a deep red colour in Mucicarmine staining. Specific treatment is not known and so the prognosis is very grave (Mamidi *et al.*, 2002; Nunez *et al.*, 2000). Necessary and appropriate cleanliness, disinfection, hygiene and sanitation measures need to be followed to prevent the infection and its zoonotic concerns (Aiello and Mays, 1998; King and Markenday, 2004; Dhama *et al.*, 2011b).

**Histoplasmosis:** *Histoplasma capsulatum* is a dimorphic fungus commonly isolated from zoo birds and less frequently affects chicken and turkeys. The soil enriched by decaying bird or bat droppings and litter with moisture flourishes the growth of this fungi (Benenson, 1995; Subramanian *et al.*, 2005; Dhama *et al.*, 2011b). This



disease is also not considered a true zoonotic disease because the reservoir is soil and not the birds. Humans acquire infection through inhalation of airborne spores. Histoplasmosis is not a contagious disease. Humans are mostly affected by asymptomatic form, but acute pulmonary influenza-like form can also be observed. The chronic form resembles tuberculosis and occurs in people above 40 years of age. The disseminated form occurs in the very young or the elderly and may have fatal consequences (Johnson and Sarosi, 1987; Wheat *et al.*, 1990; Mitchell, 1992; Dhama *et al.*, 2011b). Patients should be treated with an amphotericin B formulation and azole formulations (Dismukes *et al.*, 1992; Wheat *et al.*, 1995; Kaufman *et al.*, 1997). The diagnosis requires cultural identification of organism and histopathological examination revealing extensive proliferation of reticuloendothelial cells containing yeast forms. Methenamine silver or periodic acid-schiff staining and histoplasmin sensitivity testing are also useful for its detection. Conventional diagnostic tests like Complement Fixation Test (CFT) and immunodiffusion along with those clinical immunoassays that uses well characterized recombinant antigens (like Enzyme immunoassays) have been given much emphasis. Recently, Polymerase Chain Reaction (PCR) targeting the ribosomal RNA gene complex and real-time PCR targeting the M specific protein are have been implied for diagnosis of *H. capsulatum* (Guimaraes *et al.*, 2006; Kauffman, 2006; Dhama *et al.*, 2011b; Highland *et al.*, 2011). Biosecurity measures and appropriate precautions along with following good hygiene and disinfection practices are necessary to prevent the disease spread within birds and to prevent the transmission to humans (Mahvi, 1970; Dhama *et al.*, 2003; Jacob *et al.*, 2011).

Discussion about fungal diseases remain incomplete without mentioning about mycotoxins that appear in the food chain of both poultry and human as a result of fungal infection of crops or poultry meat as they can resist degradation. However, many international agencies are trying to achieve universal standardization of regulatory limits for mycotoxins in poultry feed as well as products (Hussein and Brasel, 2001). Moreover, the use of probiotics require a special mention instead of relying on simply antifungals, as they can efficiently attack and disrupt the fungal cell wall structures and poison their metabolic pathways, thereby inhibiting their growth. If we consider the practical application of probiotics, they significantly reduce the level of mycotoxins (Trias *et al.*, 2008). Advances in molecular diagnostic tools and techniques along with novel emerging therapies need to be exploited to their full potential

for timely diagnosis and effective treatment of fungal diseases in poultry (Dhama *et al.*, 2010, 2013).

## CONCLUSION AND FUTURE PERSPECTIVES

“Prevention is better than cure” is the best policy to be adopted for fungal/mycotic disease problems in poultry. No vaccine exist for any of the fungal diseases of poultry, therefore the timely adoption of good management practices, strict biosecurity, effective disease diagnosis and suitable preventive measures along with necessary treatment with appropriate chemotherapeutic agents are the only elements to have a check and control the fungal diseases of poultry. Apart from the fungal infections, mycotoxins are also of major concern as they are the leading cause of immunosuppression in birds, lowering their resistance level to various viral and bacterial diseases and increased mortality. Thus, a holistic approach is required to combat the adverse effects of mycotoxins and alleviate their adverse effects on high economic returns from the poultry production. This requires regular surveillance and monitoring of important mycotoxins with the use of conventional as well as modern diagnostics. Emphasis is also need to be given for implementing suitable prevention and control strategies for checking mycotoxin development and contamination in feeds and their intoxication in poultry along judicious approach in the use of probiotics.

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